Water channel proteins and their role in lens optics and cataract

The eye lens provides one-third of the optical power of the human eye and has the capacity to alter its focus for a range of distances in eyes below the sixth decade of life. It also offers a high level of image quality because of its gradient index structure. The ability to alter focus decreases with age as does the amount and wavelength of light transmitted and the causal factors involved in these losses of function, whilst not conclusively clear, have been linked to changes in the lens proteins. These proteins, the crystallins, are distributed in varying concentrations within long fiber cells that accrue in layers over existing tissue. This concentration gradient creates a gradient of refractive index or GRIN with the highest refractive index in the center gradually decreasing towards the periphery. With age, the proteins alter in their formation and structural relationship with water and this can be detrimental to transparency of the lens, to the GRIN profile and ultimately can lead to cataract.

There is another class of proteins that are less prevalent in the lens, the aquaporins, but have been increasingly recognized as potentially key to lenticular maintenance of water and nutrient transportation [1]. These are membrane proteins and three types of aquaporins are expressed in the human lens: aquaporin 0: located in fiber cells of the lens and a major constituent of cell membrane proteins [1]; aquaporin 1: found in the lens epithelial cells [1] which are adjacent to the anterior surface of the lens capsule within which the lens sits and aquaporin 5 which has been detected in the outer layers of the lens, in the lens epithelium and in mature lens cells [1,2]. Aquaporin 0 proteins also have sub-classes: aquaporin 0a (Aqp0a) and aquaporin 0b (Aqp0b). The former has been shown to contribute to maintaining lens structure by stabilizing the sutures in the anterior lens in older lenses and keeping the nucleus centralized [3]. These structural factors are important for lens optics. However, it is not known whether and how Aqp0a and Aqp0b may to the development of the GRIN profile in the lens.

Recent research conducted at SPring-8 investigated optical properties of lenses with loss-of-function deletions of *aqp0a* and/or *aqp0b* in zebrafish generated using CRISPR/Cas9 gene editing [4]. Mutations considered were single *aqp0a*$^{-/-}$ and *aqp0b*$^{-/-}$ mutants as well as double *aqp0a*$^{-/-}$/*aqp0b*$^{-/-}$ mutants and wild type lenses matched for age. Samples were taken from very early embryonic life to elderly adult stages. The refractive index of each of the lenses was measured using X-ray phase tomography based on X-ray Talbot interferometry which is located at SPring-8 **BL20B2** [4].

Opacities in the lens, manifesting as disruptions to the GRIN profile were seen in lenses from all the aquaporin 0 mutants: *aqp0a*$^{-/-}$ and *aqp0b*$^{-/-}$ and double mutants *aqp0a*$^{-/-}$/*aqp0b*$^{-/-}$ as well as in lenses from very old wild type zebrafish. However, the opacities varied depending on the mutation or whether the lens was from an old wild type zebrafish. Single *aqp0a*$^{-/-}$ mutants had asymmetric GRIN profiles and a shift of the nuclear part of the GRIN towards the anterior of the lens (Fig. 1). In contrast, single *aqp0b*$^{-/-}$ mutants showed no difference in their GRIN profiles to those from wild type lenses in developing and young samples although disruptions to the GRIN were seen in very old *aqp0b*$^{-/-}$ mutant lenses (Fig. 2) and these were similar to features observed in wild type lenses of advanced age. In lenses from zebrafish with double *aqp0a*$^{-/-}$/*aqp0b*$^{-/-}$ mutations, the refractive index magnitudes in the GRIN profiles were lower than in wild type lenses of similar ages and dips in the refractive index were seen in the central plateau region of the GRIN (Fig. 3).

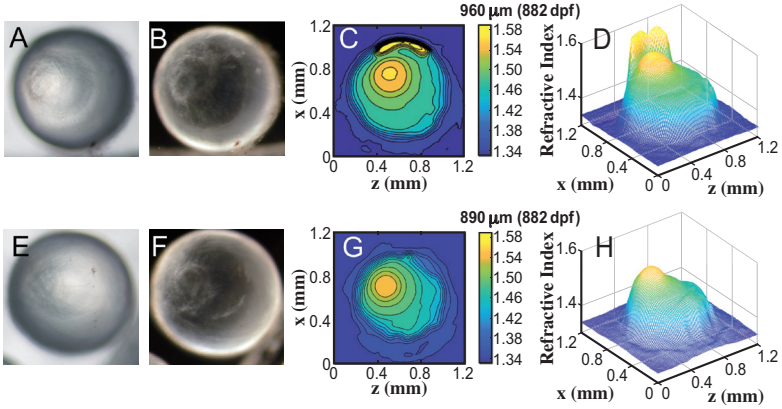

Fig. 1. Anterior polar opacity and asymmetric GRIN profiles in *aqp0a*$^{-/-}$ lens pairs: (A,E) show bright field images and (B,F) dark field images from zebrafish lenses aged 882 days post-fertilization with the corresponding 2D contours in (C,G) and 3D mesh plots of refractive index in (D,H).

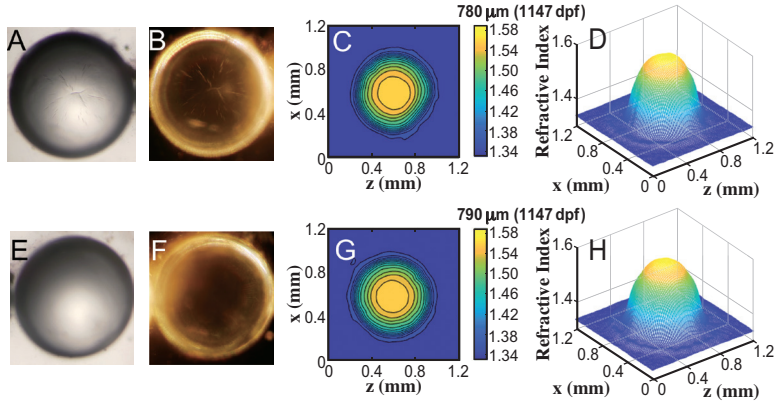

Fig. 2. Spoke cataract and disturbances to GRIN profiles in an old *aqp0b*$^{-/-}$ lens pairs: (A,E) show bright field images and (B,F) dark field images of lenses of a zebrafish aged 1147 days post-fertilization with the corresponding 2D contours in (C,G) and 3D mesh plots of refractive index in (D,H).

As the lens has no blood vessels, it is entirely reliant on circulation of water to ensure delivery of nutrients and waste removal [5]. This requires a gradient of hydrostatic pressure. The exact contribution of aquaporin 0 to maintaining the pressure gradient is as yet unclear, but the investigations conducted at SPring-8 confirms that this aquaporin has a vital role in ensuring the proper formation of the GRIN and hence is likely to have some impact on the pressure gradient. This research supports the previous findings that Aqp0a [3] plays a role in maintaining centralization of the lens nucleus and therefore symmetry of the GRIN profile. In double mutants, the refractive index magnitude in the center of the GRIN profiles was lower than age-matched wild type lenses, indicating that the high concentration of crystallin proteins critical for the appropriate magnitude of refractive index at the lens center requires presence of at least one of the two subtypes of aquaporin 0. Although aquaporins, as membrane proteins, are not involved in synthesis of the cytosolic crystallin proteins, any changes in water transport resulting from aquaporin absence could cause alterations in crystallin protein-water ratios in the lens center and hence affect the magnitude of refractive index.

The findings of this research indicate that aquaporins have an important role in optics of the lens by creating and maintaining its GRIN. There appear to be regional specificities in the roles of Aqp0a and Aqp0b. Whilst the presence of Aqp0a is needed in the peripheral lens to ensure a symmetrical GRIN profile, its role cannot be compensated by Aqp0b. However, in the lens center, Aqp0b can replace Aqp0a to ensure that the central part of the GRIN is maintained.

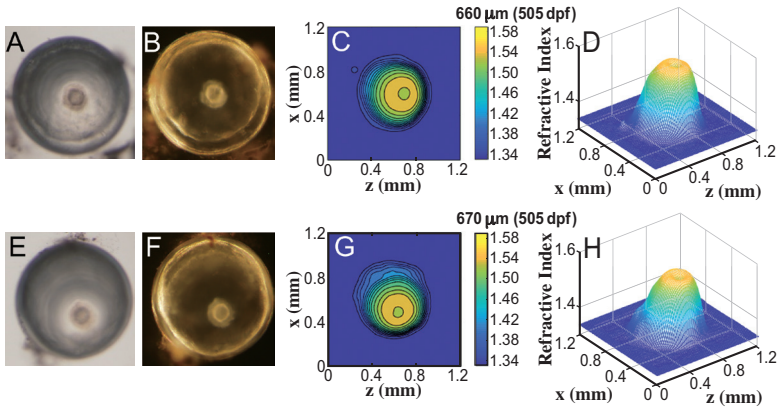

Fig. 3. Nuclear opacity and central dip to GRIN profiles in double *aqp0a*$^{-/-}$/*aqp0b*$^{-/-}$ lens pairs: (A,E) show bright field images and (B,F) dark field images of lenses of a zebrafish aged 505 days post-fertilization with the corresponding 2D contours in (C,G) and 3D mesh plots of refractive index in (D,H).

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